# Comment on "A method to measure cellular adhesion utilizing a polymer micro-cantilever" [Appl. Phys. Lett. 103, 123702 (2013)]

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In their recent report, Gaitas and colleagues[1] describe an approach to measure forces in the micro-Newton range and its possible use on cell adhesion measurements in tumor biology. Although this is technically a fascinating and new method to circumvent the major problems of classical, laser-based atomic force microscopy (AFM), including the possibility of laser-free measurements in opaque liquids, the experimental example that the authors used may not support all their conclusions, especially with respect to measuring cell adhesion and tumor progression. They use a micro-cantilever, coated only with 3,3′-dithiobis[sulfosuccinimidyl-propionate] (DTSSP), a cross-linker which can covalently but nonspecifically bind to NH2-groups of all proteins exposed on a cell membrane. The authors conclude that they measure cell adhesion, but in this example, it remains unclear what really happens when the cell detaches from the binding cantilever. There are several possibilities: (1) all the covalent bonds between DTSSP and membrane proteins rupture (which is probably assumed by the authors, but very unlikely due to the covalent nature of the bonds); (2) the membrane proteins are pulled out of the membrane; (3) all the binding DTSSP linker molecules break; (4) the membrane disrupts with parts of the membrane remaining stuck to the cantilever; or (5) a combination of several or all of the above options. Such measurements may perhaps be useful to characterize mechanical properties of living cells and may be used for further studies of tumor cell behavior, including epithelial-mesenchymal transition (EMT), but they may not accurately reflect physiological aspects of cell-cell adhesion. In similar adhesion studies with AFM, others have functionalized different cantilevers with specific ligands or counter-receptors, or even with a whole cell, to probe against living cells (reviewed in Ref. 2). As mentioned in one of the authors' references,[3] DTSSP can be used as a linker, for example for ConA, which then can reversibly bind to structures on the cell surface, but DTSSP alone generally does not appear to be used for cell adhesion studies.

It will be interesting to see how the authors' system develops further, especially if the micro-cantilever was to be functionalized with relevant cell adhesion receptors or another cell as a probe, and to compare this new laser-free method with existing AFM and other cell adhesion approaches.

[1]A. Gaitas, R. Malhotra, and K. Pienta, Appl. Phys. Lett. **103**, 123702 (2013).
[2]R. H. Eibl, "Single-molecule studies of integrins by AFM-based force spectroscopy on living cells," in *Scanning Probe Microscopy in Nanoscience and Nanotechnology*, edited by B. Bhushan (Springer, 2013), Vol. 3, pp. 137–169.
[3]A. Chen and V. T. Moy, Biophys. J. **78**(6), 2814–2820 (2000).

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